

# Design and characterization of a scalable airlift flat panel photobioreactor for microalgae cultivation

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Abstract: A novel flat panel photobioreactor prototype with bulk liquid flow driven by an external airlift was designed, modeled and experimentally characterized for the purpose of developing scalable industrial photobioreactors. Baffles were built inside the flat panel part of the reactor, directing the liquid bulk flow in a serpentine way, and the external airlift drove the liquid flow and facilitated gas mass transfer. The gas holdup, liquid flow velocity and oxygen mass transfer of this prototype were experimentally determined and mathematically modeled, and the performance of the reactor was tested by cultivating two species of microalgae, *Scenedesmus obliquus* and *Chlorella sorokiniana*. The model-predicted trends correlated well with experimental data, indicating that the reactor might be scaled up using these models. A high cell concentration of *Chlorella sorokiniana* was achieved under controlled indoor cultivation conditions although serious biofouling occurred in the case of *Scenedesmus obliquus* cultivation. The results favor the possibility of scaling up the reactor to industrial scales, based on the models employed, and the potential advantages and disadvantages of the reactor are discussed regarding this industry-oriented photobioreactor configuration in comparison with current industrial photobioreactors.

## 1. Introduction

The potential of microalgae to produce biofuels or other products has been under intensive exploration in recent years (Haag 2007; Chisti 2007; Stephens et al. 2010; Wijffels et al. 2013). One of the technical challenges is to cut down the capital and operational costs of microalgal production systems (Wijffels and Barbosa 2010; Stephens et al. 2010). Although many cultivation systems have been proposed and studied, mostly in laboratory settings, for microalgal biomass production, only raceway ponds, tubular photobioreactors and flat panel photobioreactors have the potential to be scaled up to industrial scales without forbidding costs (Norsker et al. 2011; Ugwu et al. 2008). However, the capital and operational cost of such systems are still too high to produce microalgal biomass as feedstock for biofuels or other low value products with currently available technologies (Li et al. 2011; Petkov et al. 2012), and thus it is still desirable to develop cost effective production systems to release the full potential of microalgal biotechnology (Acién et al. 2012).

Although raceway ponds are believed to be the most inexpensive systems for the industrial autotrophic cultivation of microalgae and might even be the only choice for microalgal biodiesel production, industrial tubular and flat panel photobioreactor systems are still expected to play a vital role in the future microalgal industry. For the production of low value products such as biodiesel or animal feed at large scales, the utilization of photobioreactors might be necessary to provide inoculum culture for raceway ponds to achieve sustainable production (Delrue et al. 2012; Lundquist et al. 2010). For high value products such as nutraceuticals or pharmaceuticals, the production system might need to exclude the utilization of raceway ponds for hygienic reasons (Ugwu et al. 2008). Thus, advancements of photobioreactor research could have a crucial impact for the growth of the microalgal industry (Chen et al. 2011; Lehr and Posten 2009).

Both tubular and flat panel photobioreactors have been scaled up to large scales yet neither of them has been shown to be clearly superior to the other one. Tubular photobioreactors can be constructed using inexpensive PVC film tubes with an external airlift made of plastic material, and the tubular part of the bioreactor can be lined on the ground without complicated supporting infrastructure. The oxygen removal and CO<sub>2</sub> addition can be both achieved through airlift modules, which are inexpensive, reliable and efficient (Molina et al. 2000; Acién et al. 2001). Due to this kind of design configuration, the material and operational costs of tubular reactors can be moderate. The industrial flat panel photobioreactors can also be constructed using low cost disposable PVC film bags, but these need to be supported by scaffolds, which can be expensive (Sierra et al. 2008). In addition, the sparging of the reactors requires larger volumes of air, which might mean higher energy consumption and CO<sub>2</sub> lost to the atmosphere. Comparing with industrial tubular photobioreactors, both the material and operational costs of the flat panel reactor can be higher per unit area, but the areal production can also be higher due to shorter light path length and vertical alignment of flat panels (Norsker et al. 2011; Janssen et al. 2003; Slegers et al. 2011).

In this research we cultivated microalgae in a flat panel photobioreactor with internal bulk liquid flow and an external airlift, which we designed and characterized. Although a similar concept was proposed and tested in 1992, it has never been fully exploited and developed for industrial photobioreactor design (Ratchford and Fallowfield 1992). In currently employed industrial flat panel photobioreactors, the air is bubbled directly from the bottom of the flat panels, and there is no liquid bulk flow in the reactor. In this way the efficiency of mixing is low, and thus the energy efficiency of the reactor is also low due to high consumption of compressed air (Leupold et al. 2013; Sierra et al. 2008). Another major problem of this kind of reactor is biofouling, which is also partially caused by inefficient mixing (Acién Fernández et al. 2013). Although some teams have reported occasionally flat panel photobioreactor designs with internal airlift for laboratory studies, most of them have never been scaled up for industrial applications (Reyna-Velarde et al. 2010; Degen et al. 2001) or have only been of the order of 30 L and used in industry in the form of multiple units (Münkel et al. 2013). External airlift pumps are widely used in tubular photobioreactor systems, they are inexpensive to build and can be easily scaled up. In order to improve the energy efficiency and enhance the mixing of the flat panel photobioreactor systems, we modified the current flat panel photobioreactor design configuration by supplying an external airlift pump and adding internal baffles to drive and direct the liquid bulk flow in the reactor. With our design, the flat panel part of the photobioreactor was configured in a way that liquid medium can display bulk flow as in tubular photobioreactors, and the oxygen removal and CO<sub>2</sub> addition is realized through an external airlift module. At the same time, the alignment of multiple flat panel reactors and the light path length of individual flat panels can be maintained similarly to current industrial flat panel photobioreactors if the new prototype were to be scaled up for industrial applications. In this way, the new bioreactor might be able to have high areal productivity, low energy consumption and less serious biofouling, and, simultaneously, the construction and operation costs can be kept low. Therefore, this airlift flat panel reactor might overcome some disadvantages of both industrial flat panel and tubular photobioreactors and lead to a novel class of industrial photobioreactor design which is superior to both of them.

## 2. Materials and Methods

### 2.1. The airlift flat panel photobioreactor

The prototype airlift flat panel photobioreactor was comprised of a flat panel and an external airlift pump, as shown schematically in Figure 1. The airlift had a riser and a downcomer both of which were attached to a gas liquid separator on their top ends. Both the riser and downcomer were attached to a T shape sparger at their bottom ends, and a perforated plate was used for air distribution in the sparger. The joint flow of air via the perforated plate sparger and of the liquid from the outlet of the flat panel started at the bottom of the riser then flowed to the gas liquid separator where the air and liquid were separated. The air flowed out, and the liquid flowed through the downcomer to the inlet of the flat panel. In the flat panel, the liquid flowed from bottom to top in a serpentine manner.

(Figure 1 to be placed approximately here)

The flat panel was made of transparent polycarbonate (PC) plates, with a surface area of 1 m<sup>2</sup> (1 m x 1 m) and an internal thickness of 3.6 cm, and was split into 9 lanes of a continuous flow chamber. The airlift module was made of plexiglass tubes and plates, with a tubular diameter of 3.2 cm and a height of 2.35 m. The probes and sensors were installed at the top of the gas liquid separator, which linked riser and downcomer at its bottom. The flat panel and the airlift modules were linked by soft translucent PVC tubes, and the total working volume of the whole reactor was about 48 L.

The photobioreactor was equipped with a NI cRIO-9074 CompactRIO data acquisition and control chassis and two modules from National Instruments Corporation (Austin, USA). The dissolved oxygen (DO) level and the pH of the cell culture could be constantly monitored by a ProfiLine Oxi 340i oxygen meter and ProfiLine pH 340i pH meter, respectively, both of which were from WTW (Weilheim, Germany). Temperature was monitored continuously thanks to an additional function of the pH meter. The data were transferred between the meters and the CompactRIO controller through a NI 9870 serial port module (National Instruments). The temperature could be controlled through automatic turning on and off of two radiating heaters facing the flat panel, and the pH could also be constantly controlled by on-off injection of CO<sub>2</sub> through the sparger at the bottom end of the downcomer. The control functions were realized using a NI 9481 relay module (National Instruments).

## 2.2. Organisms and cultivation conditions

The microalgal strain *Chlorella sorokiniana* SAG 211-32 was from the SAG Culture Collection of Algae (Göttingen, Germany), and *Scenedesmus obliquus* CCAP 276-2 was from the Culture Collection of Algae and Protozoa (Oban, UK). The average surface light intensity of the flat panel was about 150 μmol photons m<sup>-2</sup>s<sup>-1</sup> (continuous illumination with fluorescent light bulbs) which was determined by a PAR quantum sensor from Skye Instruments (Powys, UK). The pH of the photobioreactor was maintained constant at 7.5 by controlled injection of CO<sub>2</sub>. The aeration rate was kept at 4 L min<sup>-1</sup> for both species, and the temperature was maintained at 22 °C. The reactor was first filled with demineralized water and sterilized by ozone for 2 h, and the stock solutions of BBM medium with three-fold sodium nitrate (Stein 1979) were pumped into the reactor through a 1.0 μm liquid filter from Sartorius AG (Göttingen, Germany). The reactor was then inoculated with axenic seed cell culture.

## 2.3. Cell density measurements

The cell culture density of samples was quantified by spectrophotometry and gravimetry. For the spectrophotometric method, the optical density (OD) of the cell culture was measured in a 1 cm light path cuvette at wavelengths of 530 nm and 680 nm using a DU 640 Beckman (Indianapolis, USA) spectrophotometer (Kliphuis et al. 2010). For the gravimetric method,

duplicate cell culture samples of 40 mL were centrifuged in Eppendorf centrifuge tubes at 2000 g for 10 min, the supernatant was carefully discarded, and the pellets of microalgae were re-suspended in 2 mL distilled water and transferred to pre-weighed 2 mL Eppendorf tubes. The samples were then centrifuged at 2000 g for another 10 min and the supernatants were again carefully discarded. Finally, the pellets were dried at 70 °C for 24 h, and the weight of the biomass was measured.

#### 2.4. Liquid flow and gas holdup measurements

The liquid flow in the photobioreactor was measured with a conductivity meter, model ProfiLine340i from WTW (Weilheim, Germany). The probe of the meter was placed on the top of the gas-liquid separator, and sodium chloride was used as a tracer. The reactor was first filled with demineralized water, and the aeration was turned on to drive the liquid flow. Then a shot of saturated sodium chloride solution, about 50 mL in volume, was introduced in the reactor at the gas-liquid separator region, and the conductivity of the liquid was constantly monitored and recorded. With the liquid circulating through the reactor, a series of peaks of conductivity values were observed, and the time interval between peaks was estimated based on the data and regarded as the time duration for the liquid to travel across one cycle. This time duration was defined as the circulation time. The superficial liquid flow velocity at the riser region,  $U_L$ , could then be calculated based on the following equation (Rubio et al. 1999):

$$U_L = \frac{4V_T}{\pi t_c d_r^2} \quad (1)$$

where  $V_T$  is the total volume of the reactor,  $t_c$  is the circulation time, and  $d_r$  is the diameter of the riser.

The gas holdup in the riser was measured by the differences in liquid level between the airlift and an external tube which was linked to an opening at the bottom level of the riser. At certain aeration conditions, the liquid levels in the external tube were compared with those in the gas-liquid separator on the top of the riser, and the height differences divided by the total height of the riser were considered the values of gas holdup in the riser (Rubio et al. 1999).

#### 2.5. Oxygen transfer measurement

The oxygen transfer was measured dynamically by a DO meter, model ProfiLine Oxi 340i from WTW (Weilheim, Germany). The photobioreactor was bubbled with pure nitrogen gas through the airlift module at a flow rate equal to the aeration rate until the DO level dropped close to zero. Then, the nitrogen gas was switched off and air switched on, after which a leap of DO level was observed and recorded. After a time interval of circular flow, another leap of DO level was observed and recorded. Equation 2 can be easily derived to estimate the oxygen transfer coefficient  $k_L a$  in the riser (Acien et al. 2001; Rubio et al. 1999):

$$k_L a = \frac{U_L}{h_r} \ln \left( \frac{C^{in} - C^*}{C^{out} - C^*} \right) \quad (2)$$

where  $U_L$  is the superficial liquid flow velocity,  $h_r$  is the total height of the riser,  $C^*$  is the saturated DO level,  $C^{in}$  is the DO level at the inlet of the riser, which is the value before the leap, and  $C^{out}$  is the oxygen level at the outlet of the riser which is the value after the leap.

## 2.6. Modeling fluid dynamics and mass transfer

The equations for modeling the photobioreactor processes were adapted from literature data on conventional tubular photobioreactor systems and cell culture fluid dynamics and applied to the operation scenarios of the flat panel photobioreactor. All the software codes were programmed in Matlab software from Mathworks (Natick, USA).

Since the liquid flow in the flat panel was driven by the airlift module, we used the previously established mathematical relationships between liquid flow velocity and gas holdup based on energy balance (Chisti 1989; Chisti et al. 1988) and developed (Molina et al. 2001) to be:

$$U_L = \left[ \frac{g \varepsilon_r h_r d_i^{1.25}}{0.3164 (u_L / \rho_L)^{0.25} L_{eq}} \right]^{4/7} \quad (3)$$

where  $U_L$  is the superficial liquid velocity in the tube,  $g$  is the gravitational acceleration constant,  $\varepsilon_r$  is the gas holdup in the riser,  $h_r$  is the height of riser,  $d_i$  is the diameter of the liquid flow channel,  $u_L$  is the viscosity of the culture broth,  $\rho_L$  is the density of culture broth, and  $L_{eq}$  is the equivalent length of the liquid flow channel. The relationship between gas holdup in the riser  $\varepsilon_r$  and superficial liquid flow velocity in the riser  $U_L$  had also been established (Zuber and Findlay 1965) semi-experimentally based on mass balance:

$$\varepsilon_r = \frac{U_G}{\lambda(U_G + U_L) + U_b} \quad (4)$$

where  $U_G$  is the superficial gas velocity in the riser,  $\lambda$  is a parameter related to the fluid dynamics in the riser, and  $U_b$  is the bubble drift velocity in the riser. In our experimental settings where the liquid flow can be characterized as one-dimensional vertically upward homogeneous bubbly flow,  $\lambda$  and  $U_b$  can be estimated by the following equations (Liao et al. 1985; Wallis 1969):

$$U_b = 1.53(1 - \varepsilon_r)^2 \left[ \frac{\sigma g (\rho_L - \rho_G)}{\rho_L^2} \right]^{1/4}, \quad \lambda = 1.0 \quad (5)$$

where  $\sigma$  is the surface tension of the liquid and  $\rho_G$  is the density of the gas phase.

The oxygen transfer in the airlift was estimated according to the same literature reports as liquid flow (Chisti et al. 1988; Chisti 1989; Molina et al. 2001; Chisti 1999), which have established the guiding principles for airlift tubular photobioreactor design. The following equation, which was developed based on fundamental geometrical concepts, was found to be valid under various aeration conditions for airlift devices (Chisti and Moo-Young 1987; Chisti 1989; Cerri et al. 2010):

$$\frac{k_L}{d_B} = \frac{k_L a (1 - \varepsilon_r)}{6 \varepsilon_r} \quad (6)$$

where  $k_L$  is the true convective mass transfer coefficient,  $d_B$  is the average air bubble diameter,  $a$  is the volumetric interfacial area, and  $k_L a$  is the volumetric mass transfer coefficient. For the air-water system, the following equation (Chisti 1989) can be used to estimate  $k_L/d_B$ :

$$\frac{k_L}{d_B} = 5.63 \times 10^{-5} \left( \frac{g D_L \rho_L^2 \sigma}{\mu_L^3} \right)^{1/2} \quad (7)$$

where  $D_L$  is the diffusivity of oxygen in water and  $\mu_L$  is the viscosity of the culture medium.

## 2.7. Microalgae growth modeling and parameter determination

To simplify analysis, we assume in our experimental conditions that the growth of microalgae is only light limited, light inhibition and oxygen inhibition are negligible, and light transmission in the cell culture obeys the Beer-Lambert law. Thus the following equations can be modified from literature to describe the cell growth in the flat panel photobioreactor (Li et al. 2003; Su et al. 2003):

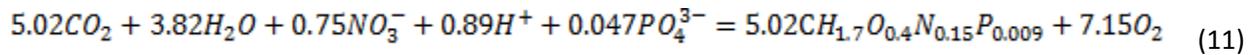
$$\frac{dX}{dt} = \mu X \quad (8)$$

$$\mu = \mu_{max} \left( \frac{I_{av}}{K_I + I_{av}} \right) - r \quad (9)$$

$$I_{av} = \frac{I_0}{L} \int_0^L e^{-K_a X l} dl = I_0 \frac{1 - e^{-K_a X L}}{L K_a X} \quad (10)$$

where  $X$  is the biomass concentration in the reactor,  $t$  is time,  $\mu$  is the specific growth rate,  $\mu_{\max}$  is maximum specific growth rate at the prevailing temperature,  $I_{av}$  is the average light intensity in the reactor chamber,  $r$  is the specific respiration rate at the given temperature,  $K_I$  is the light saturation constant,  $L$  is the thickness of the reactor chamber,  $I_0$  is the incident light intensity, and  $K_a$  is the mass specific light absorption coefficient.

We estimated  $\mu_{\max}$ ,  $r$  and  $K_I$ , which are intrinsic properties of a given microalgal strain at a defined temperature, for the strain *Chlorella sorokiniana* SAG 211-32 that we used in this research, using an OxyLab Plus System from Hanatech Instruments Inc. (Norfolk, UK) and under the same temperature and nutritional conditions as described in Section 2.2. The oxygen levels of the microalgae cell culture in the chamber under different levels of light illumination were monitored, and the rates of oxygen production were thus derived, based on the rate of oxygen level changes (Huang et al. 1998). The specific oxygen production rates were transformed into biomass growth rates, according to the chemical stoichiometry Equation 11 listed below (Munoz and Guieysse 2006):



The growth rates were then fitted with light intensity levels to get the values of  $\mu_{\max}$ ,  $r$  and  $K_I$ , based on Equation 9. The value of  $K_a$  is estimated from the light absorption levels with different cell concentrations of *Chlorella* culture, based on the Beer-Lambert law. The values of the above parameters are tabulated in Table 1.

(Table 1 to be placed approximately here)

### 3. Results and Discussion

#### 3.1. Characterization of fluid dynamics in the reactor

The fluid dynamics in photobioreactors is of primary importance as in any other bioreactor type because the turbulence affects cell physiology, keeps the cells in suspension, mixes nutrients, and facilitates gas and heat transfer. In fact, advanced engineering tools such as Computational Fluid Dynamics (CFD) have already been applied in photobioreactor design and development (Bitog et al. 2011). However, to estimate the most basic parameters of fluid dynamics in airlift photobioreactors, such as liquid flow velocity and gas holdup, some semi-empirical equations might be both sufficient and less onerous (Znad et al. 2004).

In this research, we employed established semi-empirical relationships, i.e., Equations 3, 4 and 5, to estimate liquid flow velocity and gas holdup and we compared experimentally measured values with the mathematically estimated trends. The experimental data fitted the model-predicted trends reasonably well, and we believe these model equations could be appropriate for the purpose of scaling up the airlift flat panel photobioreactor. The liquid flow pattern in the

riser of the airlift could be categorized as bubbly flow regime by visual observation, based on the near homogeneity of the bubbles and their small size compared to the tube diameter (Shaikh and Al-Dahhan 2007). The observation could hold to be true for air flow rates up to 5 L min<sup>-1</sup> (equivalent to superficial velocity up to about 0.1 m s<sup>-1</sup>), above which slug flow would take place. Thus we used fluid dynamic equations that characterize bubbly flow regime to model the liquid flow and gas holdup in the photobioreactor, as described in Materials and Methods. Figure 2 shows model-predicted and experimentally measured data of the gas holdup in the riser of the airlift, and Figure 3 shows the superficial liquid flow velocity data in the riser together with model predictions. Considering the fact that in both figures the experimental data were fitted with only one fitting variable,  $L_{eq}$ , the equivalent length of the pipe, reasonably good fittings were obtained. Although the fitting of the experimental data showed some deviation from the model-predicted gas holdup at lower aeration conditions (Fig. 2), the fitting of the experimentally obtained data of liquid flow velocity corresponded well with the model curve from low to high aeration conditions (Fig. 3).

The fitted value of  $L_{eq}$  was 15.0 m equivalent length of tube with an inner diameter of 32 mm, and this number was close to the value of the actual experimental settings. The riser and downcomer were 2.35 m high (together = 4.7m in total length), and the PVC pipe connecting the flat panel and airlift modules was, in total, about 5.0 m in length. The two tees at the bottom of the riser and the downcomer were estimated to total 3.2 m in equivalent length (Green and Perry 2007), and thus the equivalent length of panel was only about 2.1 m. If we consider that the actual conduit length of all the panel lanes was about 9 m and there were 8 return bends, the estimated equivalent length of the panel seemed shorter than expected. This can be explained by the fact that the hydraulic diameter of the panel lane was 1.7 times more than the tube.

(Figure 2 to be placed approximately here)

(Figure 3 to be placed approximately here)

### 3.2. Estimation of oxygen transfer in the riser

The estimation of oxygen mass transfer is crucial in designing photobioreactor systems, especially for large scale industrial applications. Oxygen buildup by photosynthesis harms microalgal growth in photobioreactors in at least two ways, though the significance of harm differs among different species and strains. First, high level of DO inhibits photosynthesis (Sánchez Mirón et al. 1999). Secondly, when combining with high levels of irradiance in outdoor conditions, oxygen induces photooxidation, which leads to serious biomass losses (Carvalho et al. 2011). Usually oxygen removal is not reported to be a problem because gas liquid transfer can be sufficient for small-scale devices used for scientific research and published in the literature. However, the accumulation of oxygen might be the primary drawback having contributed to failures of large-scale outdoor photobioreactor systems (Sánchez Mirón et al.

1999). Thus, engineering solutions for oxygen removal are important for photobioreactor design and development and should even be customized for specific species or strains of microalgae.

Numerous factors can affect the oxygen transfer in three-phase flow airlift devices, and accurate estimation of the oxygen volumetric mass transfer coefficient  $k_L a$  using a variety of available equations can be difficult for new prototypes like the airlift used in this research. Here we employed equations that had been used by other researchers in situations similar to ours (Molina et al. 2001). Both the model-estimated values and experimentally measured data for  $k_L a$  are shown in Figure 4. It appears that at very low aeration rates there is a certain deviation of the model estimation from the experimental data, however at middle to high aeration rates the predictions are reasonably accurate. Considering the fact that in most cases high oxygen transfer and mixing is desirable, we believe that Equations 6 and 7 may be precise enough to estimate oxygen transfer for the analysis and scale up of this photobioreactor type. It is worth mentioning here that  $k_L a$  was measured in an air-water system in the absence of any solid. The actual  $k_L a$  for a culture broth with microalgal cells and ingredients such as antifoams or other macromolecules might differ somewhat from our measurements and predictions. However, again, we do not believe that considering those details is as crucial for the purpose of photobioreactor design, especially given the generally dilute nature of algal cultures and the simple mineral media used. Thus, the approach employed here would be reasonably suitable for the purpose, considering the fact that many photobioreactors have been devised quite empirically instead of being designed on the basis of engineering principles.

(Figure 4 to be placed approximately here)

### 3.3. The cultivation of microalgae in the reactor

*Scenedesmus obliquus* and *Chlorella sorokiniana* were cultivated in the airlift flat panel photobioreactor to test the reactor performance. The cultivation of *Scenedesmus* was hampered by a very slow pace in the development of a suspension cell culture accompanied by serious biofouling, as shown in Figure 5A. The biofouling could be primarily explained by the low turbulence in the flat panel part of the reactor. The highest turbulent flow at the flat panel region could only reach a Reynolds number of about 5500 at the maximum aeration rate within the bubble flow regime in the riser region. This hypothesis was supported by the fact that biofouling was much less serious in the area where high turbulence would occur as shown in Fig. 5A. To obtain further support for the hypothesis, we cultivated the exact same *Scenedesmus* strain in an airlift tubular photobioreactor that was operated at high turbulent flow conditions, and we observed that the suspension cell culture could be developed to high concentrations without biofouling on the reactor's inner wall surface (data not shown).

(Figure 5 to be placed approximately here)

Although it was possible to raise the liquid flow velocity and thus the Reynolds number by either increasing the height of the riser or the diameter of the tubing system, we decided to cultivate another species, *Chlorella sorokiniana*, in place of *Scenedesmus obliquus* in this reactor. Increasing the height of the riser would increase the liquid pressure at least at the bottom of the riser and thus increase the inlet air pressure, and increasing the tubing sizes would increase the light dark zone of the reactor volume and capital cost. We expected that *Chlorella cells* were smaller in size and more spherical than *Scenedesmus*, and at the same time *Chlorella* did not have spines, which we thought also to be a factor contributing to the serious biofouling of *Scenedesmus*.

The cultivation of *Chlorella*, under the conditions defined in the Materials and Methods section, was successful in the reactor, and an axenic homogeneous cell culture could be developed to high cell concentrations, as shown in Figure 5B. During the whole cultivation process, the DO level at the inlet of the flat panel could be kept well below 120% of air saturation, which justified our assumption of no oxygen inhibition in the light-limited growth model (section 2.7). The average light intensity on the surface of the reactor was about 150  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ , and the biomass yield on light energy ranged from 0.2 g mol photons<sup>-1</sup> to 0.6 g mol photons<sup>-1</sup>, which was comparable with data reported for the same microalgal species in a flat panel reactor (Kliphuis et al. 2010). The OD680/OD530 ratio was constant at about 1.2 for all the samples taken during the whole growth period, which indicated that pigment levels were marginally, if at all, affected by the average light intensity in the reactor (Kliphuis et al. 2010). A typical growth curve and the model-predicted growth are shown in Figure 6. The close match of the model-predicted growth and measured biomass and OD data indicated that microalgal growth in the reactor could be described by the light-limited growth models presented in the Materials and Methods section. The light-limited growth of microalgae indicated the mass transfer of the reactor was adequate. In conclusion, the airlift flat panel photobioreactor enabled to develop axenic *Chlorella sorokiniana* cell culture to adequate biomass concentrations with controlled pH, light input, DO, and temperature conditions.

(Figure 6 to be placed approximately here)

#### 3.4. The scalability of the airlift flat panel photobioreactor

The scale up of photobioreactors constitutes a major challenge (Molina et al. 2000; Janssen et al. 2003). Just like bioreactors for heterotrophic microorganisms, photobioreactors need to be scaled up to cut down capital and operation costs. However, the scale up of photobioreactors is confronted with two major constraints. First, upon increase in the photobioreactor volume, the light penetration becomes an issue due to the mutual shading of the cells. The light path in the cell culture should not exceed about ten centimeters to prevent a large portion of culture falling into the dark zone of the reactor, where only respiration instead of photosynthesis occurs. Secondly, for closed transparent vessels with a short light path, such as long tubes, the

accumulation of oxygen produced by microalgae photosynthesis limits the length of the vessel for a single photobioreactor device (Molina et al. 2001).

The present airlift flat panel photobioreactor should be scalable to large scales for industrial applications. The scale up of the reactor volume can be realized by enlarging the surface area and specifically by the elongation of the individual flowing lanes inside of the flat panel portion without changing the light path. In this way the light path is maintained, and thus the light penetration can be kept constant for the scaled up reactors. The light-dependent growth would not be changed, and similar light utilization efficiency would be expected, comparing with small scale reactors of this type or, generally, with conventional flat panel reactors. The airlift portion can also be scaled up for O<sub>2</sub> removal or CO<sub>2</sub> addition similarly to the scale up of airlift tubular photobioreactors (Molina et al. 2001). If we imagine the flowing lanes of the flat panel module as transparent tubes, the airlift flat panel reactor would share a similar liquid flow pattern as airlift tubular photobioreactors. The O<sub>2</sub> accumulated by photosynthesis while the cell culture circulates through the flowing lanes would be stripped out at the riser of the airlift, and the CO<sub>2</sub> consumed would be replaced by injecting CO<sub>2</sub> at the bottom of the downcomer (Rubio et al. 1999).

The scale up and engineering of the airlift flat panel photobioreactor can be based on the equations tested in this research. Similarly to airlift tubular photobioreactors, the size of the reactor will also be limited by the oxygen removal capacity of the external airlift module (Molina et al. 2001). Since the oxygen removal capacity can be estimated by Equations 6 and 7, the size limits of the flat panel can be estimated, given the lighting conditions and provided that the relevant physiological data of the microalgal species concerned are known. The liquid flow velocities and turbulence levels can also be estimated, using Equations 3, 4 and 5. Putting all the predicted parameters together, the geometry of the scaled up airlift flat panel photobioreactor and the productivity of the microalgal species cultivated in the reactor can be estimated, provided that the environmental conditions and growth characteristics of the microalgal species are known (Slegers et al. 2011; Quinn et al. 2012).

As we discussed above, the size of the reactor is limited by the oxygen removal capacity of the airlift module. To scale up the reactor, we can first scale up the airlift module, and then we can define the volume and configuration of the panel on the principle of avoiding prohibitive oxygen levels. For convenience of operation and referring to the literature (Acien et al. 2001), we scale up the airlift to a configuration of 2.0 m high and 0.1 m in diameter. Assuming the total aquatic equivalent length the reactor to be roughly 80 m long and 0.1 m in diameter and according to Equation 3, 4, 5, 6 and 7, the gas holdup, superficial liquid flow velocity and  $k_La$  can be predicted versus the aeration rates (shown in SFigures 1, 2, and 3 in supplementary materials) with the highest aeration rate at 50 L min<sup>-1</sup>, which is still in the bubbly flow regime (tested by experiments, data not shown). On the one hand, if the upper limit of DO concentration of the incoming flow cell culture to the airlift is decided (which can be a design

parameter related to certain microalgal species), the maximal oxygen removal capacity of the airlift can be further estimated, according to Equation 2. For example, if the incoming DO level of this scaled up airlift is 20 mg L<sup>-1</sup>, the outflow DO could reach as low as 16.1 mg L<sup>-1</sup> when the aeration is at the upper limit of bubbly flow regime (shown in Figure S4 of supplementary material). Considering the superficial liquid flow velocity and diameter of the airlift tube, the maximal oxygen removal capacity is about 10.5 mg s<sup>-1</sup> by the airlift module (shown in Figure S5 of supplementary material). On the other hand, if we can assume that the highest production of the flat panel reactor occurs at a surface light intensity of 1000 μEm<sup>-2</sup>s<sup>-1</sup> (considering the outdoor light conditions and potential light inhibition effects, Sierra et al. 2008), the maximal productivity would occur at a biomass concentration of about 1.8 g L<sup>-1</sup> for *Chlorella sorokiniana* used in this research, and the maximal productivity would be about 0.033 g L<sup>-1</sup> h<sup>-1</sup> for a 10 cm thick flat panel reactor (shown in Figure S6 of supplementary material), according to Equations 8, 9, and 10 and using parameters in Table 1. Thus, based on the stoichiometric relationship of O<sub>2</sub> and biomass established in Equation 11, the airlift module would be sufficient for a total reactor volume of about 520 L. The airlift module and connecting tubing might occupy as much as 40 L, and a 480 L panel can be attached to the airlift, i.e. 0.1 m in width, 1.0 m in height and 4.8 m in length. The flat panel can be further divided into 10 (or 9) lanes to make bulk flow possible in that panel, the liquid flow velocity and Reynolds number are reduced to the levels appropriate for microalgae growth, and at the same time homogeneous mixing of the cell culture is guaranteed. The total equivalent length of the reactor can also be roughly 80 m in length and 0.1 m in diameter.

The maximal power consumption of the above design can be easily predicted or derived, i.e. 16.3 W for 520 L or 31.3 Wm<sup>-3</sup>. This number is slightly lower than that reported for some flat panel reactors (Sierra et al. 2008; Qiang et al. 1998), which might be explained to some degree by the high turbulence caused by bubbles in the airlift and/or the sparger design. It is difficult to predict the productivity of the reactor due to lack of outdoor cultivation data, but it should be reasonable to expect that it would yield a value similar to that of traditional flat panel reactors of similar sizes. The reactor can be further scaled up, either by enlarging the diameter or increasing the height of the airlift tube, to enhance the oxygen stripping capacity and thus the reactor volume. For example, if the diameter of the airlift module increases twofold and the superficial liquid and gas velocity are kept the same as in the above design example, the oxygen removal capacity could rise as much as fourfold. The water head provided by the airlift can be almost proportionally increased by increasing the height of the airlift. The dividers inside the panel can be also adjusted appropriately in accordance with the cell culture's requirements in terms of flow resistance and Reynolds number. Thus, there should not be any major limitations in reactor sizing except for the characteristics of the materials of construction, especially with respect to the resistance to the pressure caused by the water in the airlift.

3.5. The novelty of the airlift flat panel photobioreactor and the justification of the design

Although numerous photobioreactor designs have been proposed especially for research purposes, most of them cannot be scaled up and are not appropriate for large scale applications. The large scale industrial photobioreactor requires large working volume, inexpensive infrastructure and easy operation to minimize the unit production cost. If the volume of the reactor is small, not only is the construction cost of reactor per unit of volume high due to the sensors and valves installed and materials used, but also operations such as inoculation and harvest can be troublesome. This concept is similar to conventional bioreactors.

The only photobioreactors that have been scaled up for industrial applications are tubular and flat panel photobioreactors due to their inexpensive setup. Both types utilize inexpensive transparent materials such as glass, plexiglass or PVC membrane with low-cost structure. Usually natural sunlight instead of artificial illumination is employed for lighting of reactors to cut down the energy consumption cost. The volume of both reactor types can be as large as several hundred liters and even up to several thousand liters. Recently flat panel reactors have drawn more attention than tubular reactors due to their high sunlight utilization efficiency, and several new flat panel reactors have been developed for industrial applications. Some reactors utilize lower cost PVC membrane replacing relatively more expensive plexiglass and use water to support the reactor and to compensate for environmental temperature changes (Morweiser et al. 2010; Wijffels and Barbosa 2010). Another example is to utilize static mixers for better sunlight exposure, in order to enhance the light utilization efficiency (Bergmann et al. 2013; Degen et al. 2001; Munkel et al. 2013).

Our design consisted in adding an external airlift and baffles to the flat panel reactor in order to reduce the energy consumption and enhance the mixing. In conventional flat panel photobioreactors the compressed air is bubbled directly from the bottom of the flat panels and the cell culture does not have bulk flow (Sierra et al. 2008). Consequently a large volume of compressed air is consumed to strip the oxygen and to mix the cell culture, and serious biofouling can occur partially due to the low level of turbulence. The added external airlift has dual functions -- one is to strip oxygen, and the other is to drive the liquid flow. Airlift pumps have advantages over mechanical pumps because they are not only inexpensive and long lasting but also impose much less mechanical stress to microalgal cells. The airlift pump can be easily scaled up according to engineering equations and correlations such as those employed in this report and can even be standardized to further decrease the cost. Thus adding an external airlift does not substantially raise the cost and change the cultivating conditions of photobioreactors. Added baffles in the flat panel part of the reactor help direct the liquid flow in a serpentine manner, which enhances the degree of mixing and turbulence. The bulk flow of the cell culture would be expected to make algal cells experience the same light regime and uniform nutrient supply, enhance the mixing and turbulence and reduce energy consumption (Zhang et al. 2013). Although it might be challenging to scale up a flat panel with baffles in the sense of maintaining the same dynamic environment at the higher scale, the recent trends in developing

plastic membrane-based flat panel reactors indicates that the cost of scale might still be affordable (Degen et al. 2001).

### 3.6. The advantages and disadvantages of the airlift flat panel reactor

The present airlift flat panel photobioreactor appears to share some advantages of both airlift tubular and flat panel photobioreactors. First, it is expected that this new reactor would have high areal production and biomass concentration levels like traditional flat panel photobioreactors because both of them share the same light penetration and distribution pattern due to similar geometries. Hence the scalability of the flat panel section can be assured as it would not affect light utilization efficiency. Secondly, the reactor could be scaled up just like airlift tubular photobioreactors because of the similar pattern of liquid flow. The equations regarding cell growth, light penetration, mixing, and oxygen removal that have been developed for flat panel and airlift tubular reactors can be reliably applied for the scale up of this kind of photobioreactor. Thirdly, the industrial photobioreactor based on this concept would be stable and inexpensive to be operated like any airlift tubular photobioreactor. Comparing compressed air consumption between the airlift flat panel and a traditional flat panel reactor (Sierra et al. 2008), it can be concluded that the former might need less compressed air for the same level of oxygen removal capacity and has a higher energy efficiency than the latter. Lastly, comparing with traditional flat panel reactors, the airlift flat panel photobioreactor should have a much higher CO<sub>2</sub> utilization efficiency. By using the external airlift, the CO<sub>2</sub> addition and O<sub>2</sub> removal are uncoupled. The CO<sub>2</sub> can be injected at the downcomer of the airlift as was done in this research, and bubbles of non-dissolved CO<sub>2</sub> would circulate with the fluid to the flat panel region for further absorption before being lost to the atmosphere (Rubio et al. 1999). In this way the residence time of CO<sub>2</sub> bubbles could be increased to tens of seconds, and CO<sub>2</sub> utilization efficiency could be substantially improved.

The airlift flat panel photobioreactor might also have some limitations. First, it might be more challenging to make flat panels with internal flow lanes in an inexpensive way at large scale. Using thin film plastic materials might be a solution to scale up the flat panel reactor, but still the supporting structure would definitely increase the cost (Dillschneider and Posten 2013). Secondly, the working volume of the photobioreactor would also be restricted by the oxygen removal ability of the airlift as in the case of airlift tubular photobioreactors. Thirdly, if the liquid flow is only driven by the airlift, the turbulence of the flow might not be high enough for some microalgal species which need high turbulence to be kept in suspension.

## 4. Conclusion

The newly designed prototype airlift flat panel photobioreactor is equipped with an external airlift pump and enables bulk liquid flow in the flat panel part. This reactor can be modeled using simple equations and should be scalable for industrial applications. Since it shares similar liquid flow pattern with the airlift tubular photobioreactor and the light penetration can be kept

constant upon scale-up as in conventional flat panel photobioreactors, this industrial design should have both higher light utilization efficiency and lower construction and operation costs. The scale-up and engineering of industrial airlift flat panel photobioreactors can be based on the mathematical equations employed in this research.

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## List of symbols

$a$	specific gas liquid interfacial area ( $\text{m}^{-1}$ )
$C^*$	saturated dissolved oxygen (DO) level ( $\text{kg m}^{-3}$ )
$C^{in}$	dissolved oxygen (DO) level at the inlet of the riser ( $\text{kg m}^{-3}$ )
$C^{out}$	dissolved oxygen (DO) level at the outlet of the riser ( $\text{kg m}^{-3}$ )
$d_B$	average air bubble diameter (m)
$D_L$	diffusivity of oxygen in water ( $\text{m}^2 \text{s}^{-1}$ )
$d_r$	diameter of the riser (m)
$d_t$	diameter of the liquid flow piping system (m)
$g$	gravitational acceleration ( $\text{m s}^{-2}$ )
$h_r$	height of riser (m)
$I_o$	incident light intensity ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )
$I_{av}$	average light intensity in the reactor chamber ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )
$K_a$	mass specific light absorption coefficient ( $\text{m}^2\text{kg}^{-1}$ )
$K_I$	light saturation constant ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )
$k_L$	convective gas-liquid mass transfer coefficient ( $\text{m s}^{-1}$ )
$k_L a$	volumetric gas-liquid mass transfer coefficient ( $\text{s}^{-1}$ )
$L$	thickness of the reactor chamber (m)
$L_{eq}$	equivalent length of the liquid flow piping system (m)
$r$	specific respiration rate at certain temperature ( $\text{h}^{-1}$ )
$t$	elapsed time during cultivation in reactors (h)
$t_c$	circulation time (s)

$U_b$	bubble rising velocity ( $\text{m s}^{-1}$ )
$U_G$	superficial gas velocity ( $\text{m s}^{-1}$ )
$U_L$	superficial liquid velocity in the tube ( $\text{m s}^{-1}$ )
$V_T$	total volume of the reactor ( $\text{m}^3$ )
$X$	biomass concentration ( $\text{g L}^{-1}$ )
$\beta$	ratio of superficial gas velocity to the total superficial velocity of gas and liquid
$\varepsilon_r$	gas holdup in the riser
$\lambda$	parameter determined by the fluid dynamics in the riser
$\mu$	specific growth rate ( $\text{h}^{-1}$ )
$\mu_L$	viscosity of culture broth ( $\text{kg m}^{-1} \text{s}^{-1}$ )
$\mu_{\max}$	maximum specific growth rate at given temperature ( $\text{h}^{-1}$ )
$\rho_G$	density of the gas phase ( $\text{kg m}^{-3}$ )
$\rho_L$	density of culture broth ( $\text{kg m}^{-3}$ )
$\sigma$	surface tension ( $\text{J m}^{-2}$ )

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Figure 1. Schematic representation of the airlift flat panel photobioreactor.

Figure 2. Gas holdup measurement and model prediction.

The dots represent experimental data, the continuous line represents the model-predicted results, and the capped vertical lines show the standard errors of the measurements.

Figure 3. Liquid flow velocity measurement and model prediction.

The dots represent experimental data, the continuous line represents the model-predicted results, and the capped vertical lines show the standard errors of the measurements.

Figure 4. Estimated and experimentally measured values of  $k_L a$  in the riser.

The dots represent experimental data, the continuous line represents the model-predicted results, and the capped vertical lines show the standard errors of the measurements.

Figure 5. Cultivation of *Scenedemus obliquus* (A) and of *Chlorella sorokiniana* (B) in the airlift flat panel photobioreactor.

Serious biofouling occurred while cultivating *Scenedemus obliquus* due to low turbulence in the flat panel part of the reactor (A). On the contrary, the reactor enabled the cultivation of *Chlorella sorokiniana* to adequate biomass concentrations under controlled conditions (B).

Figure 6. Cell growth of *Chlorella sorokiniana* in the airlift flat panel photobioreactor.

The diamonds are OD measurement data, the triangles are dry weight data, and the standard errors are shown by capped vertical lines. The continuous line represents the model-predicted growth data.

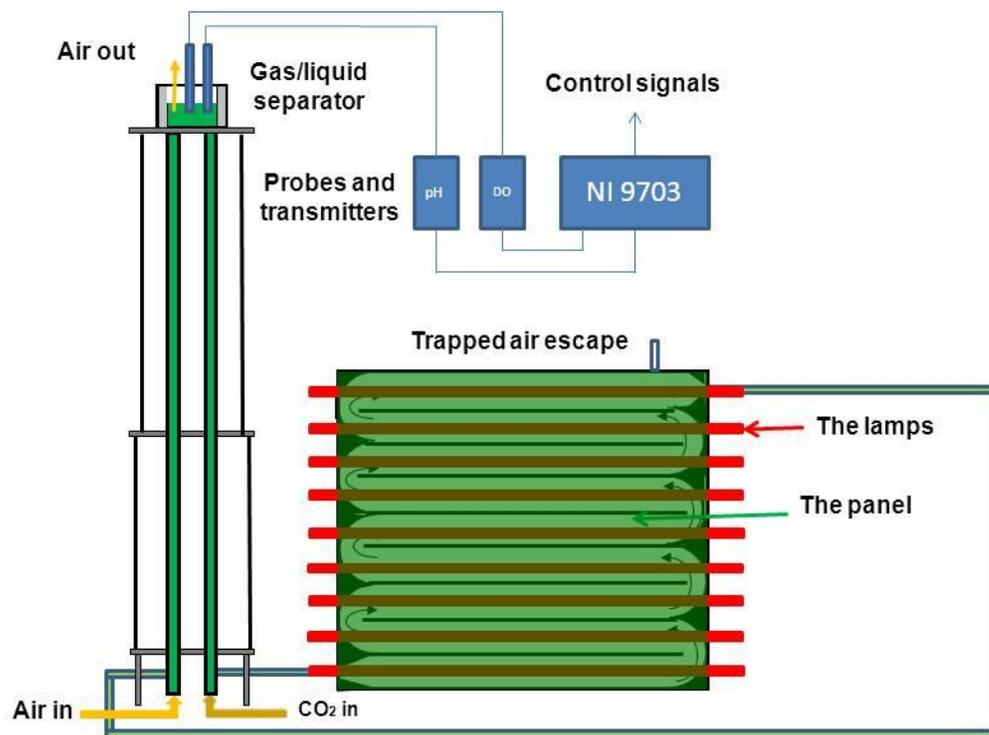


Figure 1.

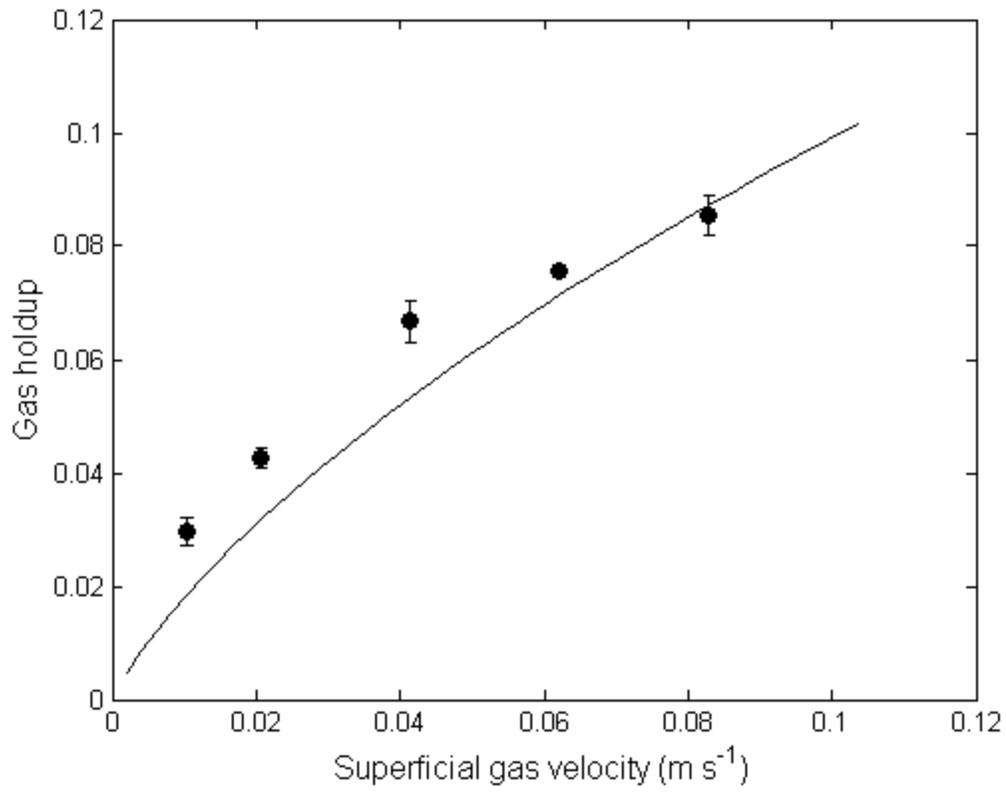


Figure 2.

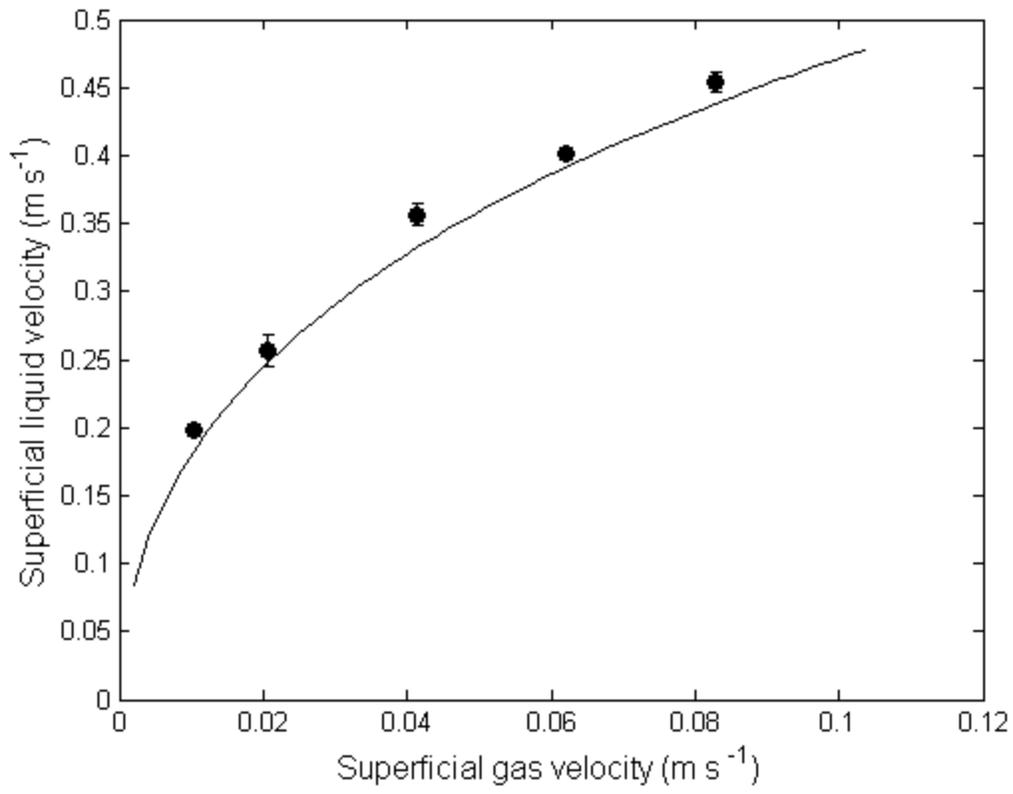


Figure 3.

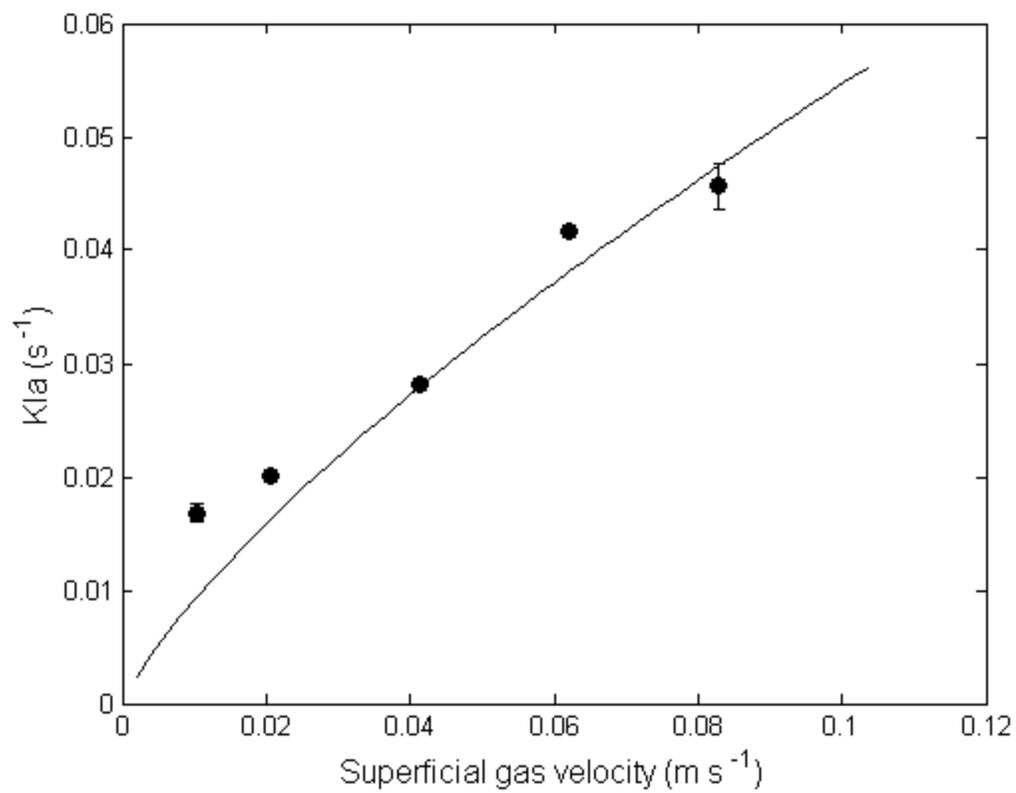


Figure 4.



Figure 5.

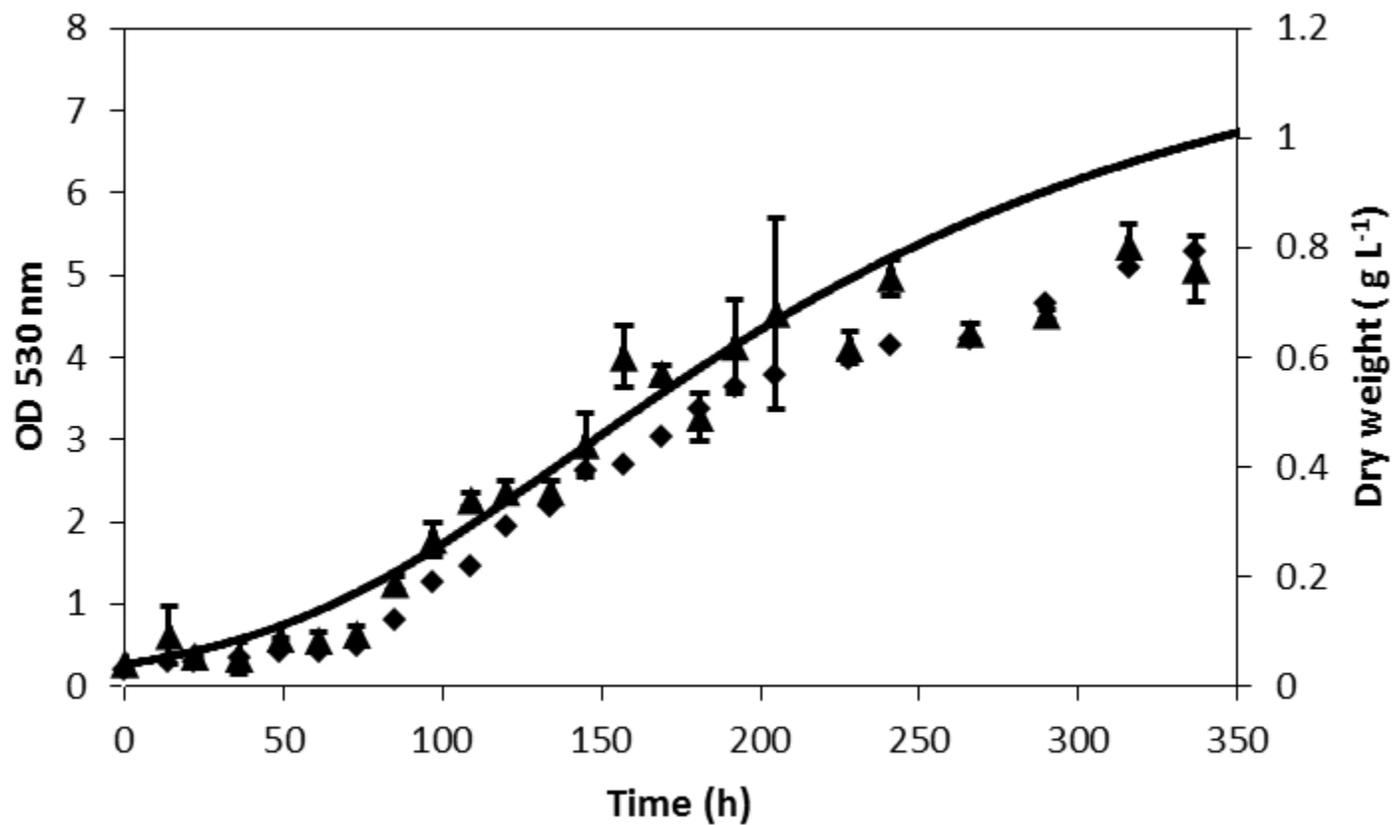


Figure 6.

Table 1 Fitted and measured microalgae growth model parameters.

Items	Description	Values	Units
$\mu_{\max}$	maximum specific growth rate	0.041	$\text{h}^{-1}$
$K_l$	light saturation constant	35.4	$\mu\text{Em}^{-2}\text{s}^{-1}$
$r$	specific respiration rate	0.01	$\text{h}^{-1}$
$K_a$	mass specific light absorption coefficient	297	$\text{kg}^{-1}\text{m}^2$